

EXHIBIT N

Nutritional Epidemiology

Dietary (n-3)/(n-6) Fatty Acid Ratio: Possible Relationship to Premenopausal but Not Postmenopausal Breast Cancer Risk in U.S. Women¹

Shelley L. Goodstine, Tongzhang Zheng, Theodore R. Holford, Barbara A. Ward, Darryl Carter, Patricia H. Owens and Susan T. Mayne²

Yale University School of Medicine and Yale Cancer Center, New Haven, CT

ABSTRACT Recent research has suggested that an increased (n-3) fatty acid intake and/or increased (n-3)/(n-6) polyunsaturated fatty acid (PUFA) ratio in the diet is associated with a lower breast cancer risk. This case-control study investigated the association between intake of (n-3) and other fatty acids and the (n-3)/(n-6) PUFA ratio and breast cancer risk. After combining data from two related case-control studies in Connecticut, we had information available on a total of 1119 women (565 cases and 554 controls). Cases were all histologically confirmed, incident breast carcinoma patients. Controls were hospital-based (Yale-New Haven Hospital study site) and population-based (Tolland County study site). Information on dietary intake was obtained through a validated food-frequency questionnaire. Standard multivariate methods were used to address the independent effects of specific fatty acids, fat classes and macronutrients on breast cancer risk. In the full study population, there were no significant trends for any macronutrient/fatty acid when comparing the highest to the lowest quartile of intake. When the analysis was restricted to premenopausal women, consumption of the highest compared with the lowest quartile of the (n-3)/(n-6) PUFA ratio was associated with a nonsignificant 41% lower risk of breast cancer [odds ratio (OR) = 0.59, 95% confidence interval (CI) 0.29, 1.19, *P* for trend = 0.09]. A higher (n-3)/(n-6) PUFA ratio was significantly associated with a lower risk of breast cancer when the data were restricted to the Tolland County (population-based) study site; OR = 0.50, 95% CI 0.27, 0.95, *P* for trend = 0.02. These results are consistent with the hypothesis that a higher (n-3)/(n-6) PUFA ratio may reduce the risk of breast cancer, especially in premenopausal women. *J. Nutr.* 133: 1409–1414, 2003.

KEY WORDS: • *breast cancer* • *case-control* • *dietary fat* • *fatty acids* • *(n-3) fatty acids*

Epidemiologic studies of fat intake and breast cancer risk have produced inconsistent results, with case-control studies suggesting an increased breast cancer risk with increasing total fat intake (1) but cohort studies generally suggesting a lack of association (2). Evidence from animal studies supports a promotional effect of dietary fat (3,4), but suggests that the type of fatty acids consumed may be important in modulating mammary carcinogenesis. More specifically, animal studies indicate that long-chain (n-3) fatty acids, present at high levels in fish oils, exert protective effects against several tumor types, including mammary tumors (5). In contrast, (n-6) fatty acids, the predominant class of unsaturated fatty acids consumed in American diets, enhance mammary carcinogenesis in rodent models (6). The animal data suggest that observational and intervention studies of dietary fat and breast cancer should thus consider type of fat.

Fish and seafood products are the primary dietary source of long-chain (n-3) fatty acids in the diet; rich sources include mackerel, herring, salmon and albacore tuna. Americans generally consume relatively little (n-3) fatty acids in the diet, but

some populations, such as the Greenland Eskimos, consume substantial amounts (7). Although their diet is high in fat, their breast cancer incidence rate is very low (8,9). Similarly, the Japanese have high fish consumption and a low breast cancer incidence rate (10–12). Ecological data suggest an inverse relationship between breast cancer incidence or mortality and consumption of fish (13,14). In an examination of analytical epidemiologic studies of fish consumption/(n-3) fatty acid intakes and breast cancer risk, Willett concluded that the analytical epidemiologic data “do not support an important association between long-chain (n-3) fatty acid intake and risk of breast cancer.” However, Willett noted that the range of intake of (n-3) fatty acids evaluated in these populations was generally quite low (15).

Another approach for examining associations between specific fatty acids and breast cancer risk is to use biochemical epidemiologic approaches. Pala et al. (16) measured fatty acids in erythrocyte membranes in a nested case-control study from a large cohort of postmenopausal women. Docosahexaenoic acid (DHA),³ a major long-chain (n-3) fatty acid, was in-

¹ Supported by grants CA62986 (National Cancer Institute) and R25 CA47883 (National Cancer Institute).

² To whom correspondence should be addressed.
E-mail: Susan.Mayne@Yale.Edu.

³ Abbreviations used: DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FFQ, food-frequency questionnaire; HCFA, Health Care Financing Administration; OR, odds ratio; YNHH, Yale-New Haven Hospital.

versely associated with subsequent breast cancer risk [odds ratio (OR) 0.48, 95% CI 0.23, 1.00 comparing highest to lowest tertile; P for trend = 0.05]. Simonsen et al. (17) conducted a case-control study of gluteal adipose tissue fatty acid content and breast cancer risk in cases and controls from five European countries. The level of (n-3) fatty acids in adipose tissue was not consistently associated with breast cancer risk across study centers. However, the long-chain (n-3)/total (n-6) PUFA ratio showed an inverse association with breast cancer risk in four of five centers (P for trend = 0.055). London et al. (18) also performed fatty acid analyses on gluteal adipose tissue from breast cancer cases and controls, and saw no association between long-chain (n-3) fatty acids and breast cancer risk. Maillard et al. (19) performed fatty acid analyses on adipose tissue from women with breast cancer (cases) and women with benign breast disease (controls), and observed inverse associations with risk of breast cancer and level of DHA, the ratio of linolenic (n-3) to linoleic (n-6) acid, and the ratio of long-chain (n-3)/total (n-6) PUFA.

To further investigate the possible relationship between (n-3) fatty acid intake and the (n-3)/(n-6) PUFA intake ratio and breast cancer risk, data were analyzed from two related case-control studies in Connecticut. The hypothesis was that total fat would be unrelated to breast cancer risk, but that (n-3) fatty acid intake and the (n-3)/(n-6) PUFA intake ratio would be inversely associated with breast cancer risk.

SUBJECTS AND METHODS

Study population. Participants in this study were women ages 31–85 y who were Connecticut residents. The cases were drawn from two sources. For the New Haven study site, eligible cases and controls were identified through the computer database system from the Yale-New Haven Hospital (YNHH) Department of Surgical Pathology and had breast-related surgery between January 1, 1994 and December 31, 1997. Controls were randomly selected from the YNHH computer database system, and had histologically confirmed benign diagnoses. For the Tolland County study site, residents of Tolland County who were diagnosed with breast cancer were identified from area hospital records by the Rapid Case Ascertainment Shared Resource of the Yale Comprehensive Cancer Center. Controls from Tolland County were population-based, and recruited by two methods. Controls <65 y old were recruited through random digit dialing methods (20); Health Care Financing Administration (HCFA) files were used to recruit controls from Tolland County ≥ 65 y old. We attempted to frequency-match the cases and controls by age, within 5-y intervals, using a 1:1 ratio by adjusting the number of controls randomly selected in each age stratum every few months. Participants were excluded if they had a prior diagnosis of cancer, excluding nonmelanoma skin cancer, or if they were not alive at the time of the interview. Certain data used in this study were obtained from the Connecticut Tumor Registry located in the Connecticut Department of Public Health. The authors assume full responsibility for analyses and interpretation of these data.

Cases were histologically confirmed, incident breast cancer patients (International Classification of Diseases 174.0–174.9). For this study, a total of 608 incident breast cancer cases were identified and completed in-person interviews. A total of 609 control patients were selected and completed in-person interviews. Of the 609 controls, 47 had a diagnosis of benign (no specific abnormality), 51 were diagnosed with fibroadenomas, 129 were diagnosed with nonproliferative benign breast disease, 177 were diagnosed with proliferative benign breast disease without atypia, and 205 were population-based controls from Tolland County who were recruited through methods described above. Controls were excluded if they were given a diagnosis of atypical hyperplasia. The study pathologist examined all of the pathological information for the breast cancer cases and controls (benign breast disease) from YNHH, as well as the breast cancer cases recruited from Tolland County.

Interviews. The Yale Human Investigations Committee and the Connecticut Department of Public Health Human Investigation Committee approved the protocol used throughout this study. Each potential participant was notified by letter and then by telephone after the approval from the subject's physician, or after being selected by random sampling. Trained interviewers went to the participant's home or another specified location to obtain written consent and conduct the interview. Each interview lasted ~90 min. Major or suspected risk factors for breast cancer and demographic information were obtained through a standardized, structured questionnaire. Risk factors included age, age at menarche, age at first birth, number of live pregnancies, race, family history of breast cancer and lactation history. A semiquantitative food frequency questionnaire (FFQ) developed by the Fred Hutchinson Cancer Research Center was used to record information about usual dietary intake in the year before interview. This questionnaire has been validated and was designed to optimize estimation of fat intake (21). Both the demographic questionnaire and the FFQ were administered by the interviewer. Case interviews were completed 3 mo after diagnosis, on average. After completion, the FFQ was sent to the Fred Hutchinson Cancer Research Center for analysis. Average daily nutrient intakes were calculated using the University of Minnesota Nutrition Coding Center Nutrient Data System database.

Statistical analyses. For this analysis, 16 participants (12 cases, 4 controls) were excluded due to an incomplete FFQ. An additional 82 participants were excluded because their estimated average daily energy intake was either <3347.2 kJ (800 kcal; 27 cases, 46 controls) or >16736 kJ (4000 kcal; 4 cases, 5 controls). Thus, the final analysis included a total of 1119 participants, 565 cases and 554 controls.

The LOGISTIC procedure in SAS version 6.12 software was used for unconditional logistic regression (SAS Institute, Cary, NC). The associations among total fat, classes of fat and specific fatty acids were modeled both as continuous variables and as quartiles of intake. Cutpoints for quartiles of intake were based on distributions in the control group. The ratio of (n-3)/(n-6) PUFA was calculated by adding the intake of all estimated (n-3) PUFA [α -linolenic acid, stearidonic acid, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and DHA] and dividing by the intake of all estimated (n-6) PUFA (linoleic acid and arachidonic acid). Odds ratios for this new continuous variable were evaluated using quartiles, with cutpoints for quartiles based on the distribution in the control group. Tests for linear trend were done by treating the quartiles as a continuous variable in the logistic models. A priori, we also specified that analyses would be performed stratified by menopausal status and by study site. The energy decomposition method was used to adjust for total energy intake (22). Differences were considered significant at $P < 0.05$.

A number of potential confounders were evaluated in multivariate models. The following potential confounders were included in the final model: age, age at menarche, age at first birth, number of live pregnancies, lactation history (months), body mass index, menopausal status (pre-, postmenopausal), race (white, nonwhite), positive family history of breast cancer in first degree relative (yes, no) and income (\$0 to $\leq \$8333$, $> \$8333$ to $\leq \$13,750$, $> \$13,750$ to $\leq \$22,500$, $> \$22,500$, or unknown).

RESULTS

Despite attempts at frequency-matching cases and controls on the basis of age, cases were significantly older than controls ($P < 0.01$) and were significantly more likely to be postmenopausal ($P < 0.01$). No significant differences were found for any other demographic factor.

Cases and controls did not differ in terms of total energy intake, macronutrient intake, fat class intake (saturated, monounsaturated, polyunsaturated) or intake of specific fatty acids (data not shown). Nutrients were then categorized by quartile and adjusted OR and tests for linear trends calculated. There were no significant trends for any nutrient (Table 1). Consumption of a higher (n-3)/(n-6) PUFA intake ratio tended to be associated with a lower risk of breast cancer (OR

TABLE 1

Adjusted odds ratios for breast cancer in women by quartile of intake of selected nutrients¹

Quartile	Intake	Cases	Controls	OR ²	95% CI	P-value ³
	g/d	%				
Total energy, kJ/d						
1	3349–5004	23.19	24.91	1.0		0.38
2	>5004–6161	24.42	25.09	1.10	0.78, 1.56	
3	>6161–7745	28.14	25.09	1.18	0.84, 1.66	
4	>7745–16747	24.25	24.91	1.15	0.81, 1.63	
Total carbohydrate						
1	≤142.9	23.01	25.09	1.0		0.42
2	>142.9–183.2	26.02	24.91	1.04	0.73, 1.50	
3	>183.2–225.5	23.19	24.91	1.29	0.87, 1.91	
4	>225.5	27.79	25.09	1.09	0.63, 1.89	
Total protein						
1	≤44.3	23.19	24.91	1.0		0.59
2	>44.3–55.8	22.65	25.09	1.15	0.80, 1.64	
3	>55.8–71.5	30.27	25.09	1.03	0.69, 1.54	
4	>71.5	23.89	24.91	1.24	0.73, 2.10	
Total fat						
1	≤42.6	21.59	25.09	1.0		0.73
2	>42.6–59.3	27.43	24.91	1.28	0.89, 1.82	
3	>59.3–78.0	26.19	24.91	1.16	0.79, 1.72	
4	>78.0	24.78	25.09	1.08	0.64, 1.84	
Total SFA						
1	≤14.8	24.42	24.91	1.0		0.83
2	>14.8–20.4	25.84	25.09	1.03	0.73, 1.47	
3	>20.4–28.3	23.54	24.91	0.97	0.66, 1.41	
4	>28.3	26.19	25.09	0.97	0.59, 1.58	
Total MUFA						
1	≤15.1	22.12	24.91	1.0		0.55
2	>15.1–21.0	26.55	25.09	1.12	0.79, 1.60	
3	>21.0–27.7	25.13	25.09	1.12	0.77, 1.65	
4	>27.7	26.19	24.91	1.17	0.70, 1.95	
Total PUFA						
1	≤9.2	23.01	25.09	1.0		0.82
2	>9.2–12.6	26.19	24.91	1.10	0.78, 1.56	
3	>12.6–16.6	25.31	24.91	1.07	0.74, 1.55	
4	>16.6	25.49	25.09	1.06	0.68, 1.64	
EPA						
1	≤0.01	24.6	24.91	1.0		0.70
2	>0.01–0.04	28.32	25.09	1.18	0.84, 1.65	
3	>0.04–0.06	25.31	25.09	1.14	0.80, 1.62	
4	>0.06	21.77	24.91	0.94	0.66, 1.34	
DHA						
1	≤0.04	24.25	25.09	1.0		0.92
2	>0.04–0.08	27.61	24.91	1.20	0.85, 1.69	
3	>0.08–0.13	25.13	24.91	1.14	0.80, 1.62	
4	>0.13	23.01	25.09	1.00	0.70, 1.44	
Ratio (n-3)/(n-6) ⁴						
1	≤0.11	27.43	24.91	1.0		0.16
2	>0.12–0.14	27.08	25.09	0.97	0.70, 1.35	
3	>0.14–0.31	22.65	24.91	0.82	0.58, 1.16	
4	>0.31	22.83	25.09	0.82	0.58, 1.15	

¹ Abbreviations: OR, odds ratio; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

² Adjusted for age, age at menarche, age at first birth, number of live births, lactation history, body mass index, menopausal status, race, family history of breast cancer and income.

³ Test for linear trend in adjusted OR for quartiles.

⁴ (Linolenic + stearidonic + EPA + docosapentaenoic + DHA)/(linoleic + arachidonic acid).

0.82, 95% CI 0.58, 1.15, $P_{\text{trend}} = 0.16$). A priori, our analytic plan included stratification of the data by menopausal status (Table 2). In premenopausal women, consumption of the highest compared with the lowest quartile of the (n-3)/(n-6) PUFA ratio was associated with a 41% nonsignificant lower risk of breast cancer (OR = 0.59, 95% CI 0.29, 1.19, $P_{\text{trend}} = 0.09$). A similar decrease in breast cancer risk was not seen in postmenopausal women (OR = 0.89, 95% CI 0.60, 1.34,

$P_{\text{trend}} = 0.48$). Although nonsignificant, an association with lower breast cancer risk was observed in premenopausal women when comparing the highest to lowest quartile of intake of the predominant long-chain (n-3) fatty acids, EPA and DHA (21 and 18% lower risk, respectively), yet similar associations were not seen in postmenopausal women. A priori, our analytic plan also included stratification of the data by study site (Table 3). Higher relative to lower intakes of the

TABLE 2

Adjusted odds ratios for breast cancer in women by quartile of (n-3) fatty acid intake, stratified by menopausal status¹

Quartile	Premenopausal, n = 311			Postmenopausal, n = 808		
	OR ²	95% CI	P-value ³	OR	95% CI	P-value
EPA						
1 (lowest)	1.0		0.65	1.0		0.80
2	0.88	0.45, 1.73		1.25	0.84, 1.87	
3	1.02	0.51, 2.03		1.18	0.78, 1.78	
4	0.79	0.38, 1.64		0.97	0.64, 1.47	
DHA						
1 (lowest)	1.0		0.70	1.0		0.94
2	0.81	0.41, 1.58		1.37	0.92, 2.06	
3	0.95	0.47, 1.92		1.24	0.82, 1.87	
4	0.82	0.40, 1.68		1.06	0.70, 1.62	
Ratio (n-3)/(n-6) ⁴						
1 (lowest)	1.0		0.09	1.0		0.48
2	1.07	0.57, 2.02		0.94	0.63, 1.40	
3	0.73	0.37, 1.43		0.83	0.56, 1.25	
4	0.59	0.29, 1.19		0.89	0.60, 1.34	

¹ Abbreviations: OR, odds ratio; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.² Adjusted for age, age at menarche, age at first birth, number of live births, lactation history, body mass index, menopausal status, race, family history of breast cancer and income.³ Test for linear trend in adjusted OR for quartiles.⁴ (Linolenic + stearidonic + EPA + docosapentaenoic + DHA)/(linoleic + arachidonic acid).

(n-3)/(n-6) PUFA ratio were associated with a significantly lower risk of breast cancer in the Tolland County (population-based) study site (adjusted OR = 0.50, 95% CI 0.27, 0.95, $P_{\text{trend}} = 0.02$), which was particularly notable in the premeno-

pausal women (OR = 0.24, 95% CI 0.06, 1.03). This effect was not observed for the YNHH (hospital-based) study site data ($P = 0.89$).

The energy decomposition method was used to adjust for

TABLE 3

Adjusted odds ratios for breast cancer in women by quartile of (n-3)/(n-6) PUFA intake and stratified by location and menopausal status¹

Quartile	YNHH ²			Tolland		
	OR ³	95% CI	P-value ⁴	OR	95% CI	P-value
All women						
n		764			355	
Ratio (n-3)/(n-6) ⁵						
1 (lowest)	1.0		0.89	1.0		0.02
2	1.11	0.74, 1.68		0.60	0.33, 1.11	
3	0.99	0.66, 1.49		0.41	0.21, 0.80	
4	1.01	0.66, 1.53		0.50	0.27, 0.95	
Premenopausal women						
n		227			84	
Ratio (n-3)/(n-6)						
1 (lowest)	1.0		0.38	1.0		0.07
2	1.46	0.71, 3.01		0.49	0.15, 1.61	
3	0.81	0.38, 1.70		0.64	0.17, 2.41	
4	0.85	0.39, 1.85		0.24	0.06, 1.03	
Postmenopausal women						
n		537			271	
Ratio (n-3)/(n-6)						
1 (lowest)	1.0		0.94	1.0		0.13
2	1.03	0.64, 1.66		0.78	0.40, 1.52	
3	1.08	0.67, 1.75		0.48	0.24, 0.99	
4	1.00	0.62, 1.62		0.67	0.34, 1.30	

¹ Abbreviation: OR, odds ratio.² Hospital-based [Yale-New Haven Hospital (YNHH)] vs. population-based (Tolland County).³ Adjusted for age, age at menarche, age at first birth, number of live births, lactation history, body mass index, menopausal status, race, family history of breast cancer and income.⁴ Test for linear trend in adjusted OR for quartiles.⁵ (Linolenic + stearidonic + eicosapentaenoic acid + docosapentaenoic + docosahexaenoic acid)/(linoleic + arachidonic acid).

total energy intake. The baseline model included all covariates, and all subsequent models also included the covariates. The second model included total energy intake as a continuous variable; the third model decomposed total energy intake into its constituents (energy from carbohydrate, fat and protein). The fourth model included energy from carbohydrate, protein and fat, with the latter decomposed into saturated, monounsaturated and polyunsaturated fat intake. A fifth model expanded on the fourth model, but decomposed energy from polyunsaturated fat into specific fatty acids (linoleic, α -linolenic, stearidonic, arachidonic, EPA, DPA, DHA). The change in the likelihood ratio statistic after each subsequent decomposition was used to determine whether any of the decompositions contributed significantly to the ability of the model to predict breast cancer risk. Results indicated that the decompositions did not significantly contribute to the ability of the model to predict breast cancer risk.

DISCUSSION

Unlike previous case-control studies, this study investigated the association between intake of (n-3) and other specific fatty acids as well as the (n-3)/(n-6) PUFA ratio and breast cancer risk in both pre- and postmenopausal women. Although not significant, when comparing the highest to the lowest (n-3)/(n-6) PUFA ratio quartile, there was a 41% reduction in the odds of breast cancer in premenopausal women, yet only an 11% reduction in postmenopausal women (Table 2). These findings differ from previous studies in several respects. One study included the (n-3)/(n-6) PUFA ratio measured in gluteal adipose tissue yet included only postmenopausal women (17); another used erythrocyte membranes to assess exposure to long-chain (n-3) fatty acids, but also included only postmenopausal women (16); and two other studies included dietary intake data for specific (n-3) and (n-6) PUFA yet did not present data stratified by menopausal status, nor was the (n-3)/(n-6) PUFA ratio presented (23,24). A strength of this study is that the (n-3)/(n-6) PUFA ratio was calculated using estimated dietary intakes of several (n-3) PUFA (α -linolenic acid, stearidonic acid, EPA, DPA, and DHA) and (n-6) PUFA (linoleic acid and arachidonic acid). The only other known case-control studies that calculated the (n-3)/(n-6) PUFA ratio are those by Simonsen et al. (17) and Maillard et al. (19), both of whom observed inverse associations with breast cancer risk. Simonsen et al. (17) reported a 30% decrease in the odds of postmenopausal breast cancer for all centers pooled when comparing the highest to lowest tertile of total (n-3)/(n-6) PUFA ratio (OR = 0.70, 95% CI = 0.38, 1.19). Maillard et al. (19) observed an even stronger inverse association that was significant for the long-chain (n-3)/total (n-6) PUFA (OR = 0.33, 95% CI 0.17, 0.66). Both of these authors used tissue levels of (n-3) and (n-6) PUFA, which are likely better predictors of breast cancer risk than dietary intakes. These studies and our own suggest the importance of estimating the (n-3)/(n-6) PUFA ratio in relation to breast cancer risk.

Another strength of the present study is that the analytical models were stratified by menopausal status. Stratification revealed that the results for premenopausal women differed from those for postmenopausal women for specific nutrients. In premenopausal women, there was a 41% nonsignificant decreased odds of breast cancer with an increasing (n-3)/(n-6) PUFA ratio, yet only an 11% decreased odds of breast cancer in postmenopausal women. Differences in breast cancer risk between pre- and postmenopausal women associated with specific fatty acids may be related to plasma estrogen levels, although the relationship between dietary fat and endogenous estrogen levels remains un-

clear (22). There is evidence that estrogen metabolism is involved in mammary carcinogenesis because the natural estrogen 17 β -estradiol stimulates neoplastic transformation (5). Grammatikos et al. (25) found that EPA, DHA and to a lesser extent, linolenic acid, inhibited the growth of the estrogen-dependent MCF-7 breast cancer cell line. Thus, a dietary intervention with long-chain (n-3) PUFA to reduce breast cancer risk may show differential results in pre- and postmenopausal women due to their levels of endogenous estrogen. The discordant findings between pre- and postmenopausal women in this and other studies necessitate stratification by menopausal status in future research examining the relationship between dietary fatty acid intake and breast cancer risk.

The limitations of this study include those inherent in case-control studies, as well as methodological issues specifically related to nutritional analyses. Case-control studies of diet and cancer are subject to recall bias when ascertaining past dietary information. Recall bias can produce differential measurement error, which can unpredictably bias OR. However, there is no reason to believe that cases would recall intakes of specific fatty acids differently from controls, and we saw no association between total fat intake and breast cancer risk in this study. Also of concern in case-control studies is generalized measurement error; this nondifferential misclassification bias would attenuate the OR toward the null, reducing study power. Greenwald (26) states that even if a strong relationship exists between dietary fat intake and breast cancer, dietary records and the FFQ may not be sufficient to quantify an association, given the likelihood of measurement error.

Other limitations of nutritional analyses include confounding of nutrients or a situation in which one or more dietary variables are subcomponents of one another, such as fat classes within total fat intake (22). To overcome this limitation, the standard multivariate method was employed in the present study to address the independent effects of macronutrients, fat classes and specific fatty acids on breast cancer risk when adjusting for total energy intake. The energy decomposition method was also used to determine whether macronutrients, fat classes and specific fatty acids had an independent association with breast cancer risk. Yet it is important to note that none of the dietary factors are measured perfectly and each has various degrees of measurement error that may influence interpretation of any multivariate model (22).

It is important to note that stratification by location revealed that there was a significantly decreased risk of breast cancer (P for trend = 0.02) for participants from Tolland County when comparing the highest to lowest (n-3)/(n-6) PUFA ratio quartile (Table 3). A similar effect was not seen for participants from YNHH (P for trend = 0.89). This finding may reflect chance or may be the result of a control group from Tolland County that was more representative of the population that produced the cases. That is, the controls from Tolland County were population-based controls, recruited through either random digit dialing methods or HCFA files. In contrast, controls from YNHH were hospital-based controls found to have benign diagnoses. The latter control group is not ideal in that fatty acid intake could be associated with benign breast disease, leading to the failure to detect an association between fatty acid intake and breast cancer risk, although Maillard et al. (19) used a benign breast disease control group and still observed inverse associations as hypothesized. Despite the results of Maillard et al. (19), we believe that the results, when stratified by study site, are less biased for Tolland County than for New Haven.

The present study adds to the current body of research with the finding that there was a nonsignificant reduction in the odds

of premenopausal breast cancer when comparing the highest to lowest (n-3)/(n-6) PUFA ratio quartile. Other studies have suggested the importance of the dietary (n-3)/(n-6) PUFA ratio, rather than absolute levels of specific classes of PUFA in relation to cancer risk (17,27). Competition occurs between the (n-6) and the (n-3) PUFA for the elongase and desaturase enzymes, yet (n-3) PUFA have greater enzyme-substrate affinities than (n-6) PUFA. It has been shown that increasing dietary intake of the (n-3) PUFA, EPA, DHA and linolenic acid, decreases the desaturation of linoleic acid, and thus, the production of arachidonic acid (5). Thus, there is increasing evidence to suggest the importance of the (n-3)/(n-6) PUFA ratio and the possible beneficial effects in reducing breast cancer risk as a result of increasing the (n-3)/(n-6) PUFA ratio. Rose and Connolly (5) suggested that dietary supplementation with (n-3) fatty acids and a concomitant overall reduction in dietary fat intake to 25–30% of total energy intake and reduction in linoleic acid intake to 5 g/d could have a substantial effect on the reduction of breast cancer risk for North American and Northern European women. Short-term dietary interventions have shown that it is possible to increase the (n-3)/(n-6) PUFA ratio in the breast adipose tissue of breast cancer patients, with the long-term goal of prevention of a second primary breast neoplasm (28). Rose and Connolly (5) recommended that long-term dietary interventions with (n-3) fatty acids should focus on women at increased risk of breast cancer and women who have had surgical treatment for breast cancer because the mechanisms of the (n-3) fatty acids do not compete with antiestrogen chemotherapies, and may enhance the antitumor activity of cytotoxic drugs (29,30). Our own research group, as well as others (31), are currently pursuing pilot intervention trials using dietary supplements of long-chain (n-3) fatty acids to study effects on biological markers of breast cancer risk in premenopausal women. Results of these trials will help to clarify the potential role of long-chain (n-3) fatty acids for breast cancer prevention.

In conclusion, our results indicate that overall fat intake and intake of specific fatty acids are unrelated to breast cancer risk. Subgroup analyses suggested that a higher (n-3)/(n-6) PUFA ratio may reduce the risk of breast cancer in premenopausal but not postmenopausal women. Findings based on subgroup analyses should be interpreted cautiously; however, dietary intake levels of (n-3) PUFA in the study were very low compared with (n-6) PUFA. Even a modest increase in (n-3) PUFA intake would increase the (n-3)/(n-6) PUFA ratio and might be a prudent approach to possibly reduce the risk of breast cancer.

ACKNOWLEDGMENTS

We would like to thank the study participants for their involvement in this project; the physicians and healthcare personnel at YNHH; Alan Kristal, Fred Hutchinson Cancer Research Center; and the Yale Cancer Center Rapid Case Ascertainment Shared Resource. Special thanks to Kumiko Iwamoto, Gwen Collman, and G. Iris Abrams of the National Institutes of Health (NIH) for guidance and support of the overall breast cancer study.

LITERATURE CITED

- Howe, G. R., Hirohata, T., Hislop, T. G., Iscovich, J. M., Yuan, J., Katsouyanni, K., Lubin, F., Marubini, E., Modan, B., Rohan, T., Toniolo, P. & Shunzhang, Y. (1990) Dietary factors and risk of breast cancer: combined analysis of 12 case-control studies. *J. Natl. Cancer Inst.* 82: 561–569.
- Hunter, D. J., Spiegelman, D. & Adami, H. (1996) Cohort studies of fat intake and the risk of breast cancer—a pooled analysis. *N. Engl. J. Med.* 334: 356–361.
- Carroll, K. K. & Khor, H. T. (1971) Effects of level and type of dietary fat on incidence of mammary tumors induced in female Sprague-Dawley rats by 7, 12-dimethylbenz[*a*]anthracene. *Lipids* 6: 415–420.
- Cohen, L. A., Thompson, D. O., Maeura, Y., Choi, K., Blank, M. E. & Rose, D. P. (1986) Dietary fat and mammary cancer. I. Promoting effects of different dietary fats on *N*-nitrosomethylurea-induced rat mammary tumorigenesis. *J. Natl. Cancer Inst.* 77: 33–42.
- Rose, D. P. & Connolly, J. M. (1999) Omega-3 fatty acids as cancer chemopreventive agents. *Pharmacol. Ther.* 83: 217–244.
- Braden, L. M. & Carroll, K. K. (1986) Dietary polyunsaturated fat in relation to mammary carcinogenesis in rats. *Lipids* 21: 285–288.
- Simopoulos, A. P. (1988) Omega-3 fatty acids in growth and development and in health and disease. Part II: The role of omega-3 fatty acids in health and disease: dietary implications. *Nutr. Today* May/June: 12–18.
- Bang, H. O., Dyerberg, J. & Hjorne, N. (1976) The composition of food consumed by Greenland Eskimos. *Acta Med. Scand.* 200: 69–73.
- Kromann, N. & Green, A. (1980) Epidemiological studies in the Upernavik District, Greenland: incidence of some chronic diseases. *Acta Med. Scand.* 208: 401–406.
- Tamura, Y., Hirai, A., Terano, T. & Yoshida, S. (1990) Clinical and epidemiological studies of omega-3 polyunsaturated fatty acids in Japan. In: *Nutritional Support in Organ Failure* (Tanaka, T. & Okada, A., eds.), pp. 89–95. Elsevier, New York, NY.
- Lands, W. E. M., Hamazaki, T., Yamazaki, K., Okuyama, H., Sakai, K., Goto, Y. & Hubbard, V. S. (1990) Changing dietary patterns. *Am. J. Clin. Nutr.* 51: 991–993.
- Wynder, E. L., Fujita, Y., Harris, R. E., Hirayama, T. & Hiyama, T. (1991) Comparative epidemiology of cancer between the United States and Japan. *Cancer* 67: 746–763.
- Kaizer, L., Boyd, N. F., Kriukov, V. & Trichler, D. (1989) Fish consumption and breast cancer risk: an ecological study. *Nutr. Cancer* 12: 61–68.
- Caygill, C. P. J., Charlett, A. & Hill, M. J. (1996) Fat, fish, fish oil and cancer. *Br. J. Cancer* 74: 159–164.
- Willett, W. C. (1997) Specific fatty acids and risk of breast and prostate cancer: dietary intake. *Am. J. Clin. Nutr.* 66: 1557S–1563S.
- Pala, V., Krogh, V., Muti, P., Chajes, V., Riboli, E., Micheli, A., Saadatian, M., Sieri, S. & Berrino, F. (2001) Erythrocyte membrane fatty acids and subsequent breast cancer: a prospective Italian study. *J. Natl. Cancer Inst.* 93: 1088–1095.
- Simonsen, N., van't Veer, P., Strain, J. J., Martin-Moreno, J. M., Huttenen, J. K., Navahas, J. F., Martin, B. C., Thamm, M., Kardinaal, A. F. M., Kok, F. J. & Kohlmeier, L. (1998) Adipose tissue omega-3 and omega-6 fatty acid content and breast cancer in the EURAMIC study. *Am. J. Epidemiol.* 147: 342–352.
- London, S. J., Sacks, F. M., Stampfer, M. J., Henderson, I. C., Maclure, M., Tomita, A., Wood, W. C., Remine, S., Robert, N. J. & Dmochowski, J. R. (1993) Fatty acid composition of the subcutaneous adipose tissue and risk of proliferative benign breast disease and breast cancer. *J. Natl. Cancer Inst.* 85: 785–793.
- Maillard, V., Bougnoux, P., Ferrari, P., Jourdan, M.-L., Pinault, M., Laviellonniere, F., Body, G., Le Floch, O. & Chajes, V. (2002) n-3 and n-6 Fatty acids in breast adipose tissue and relative risk of breast cancer in a case-control study in Tours, France. *Int. J. Cancer* 98: 78–93.
- Hartge, P., Brinton, L. A. & Rosenthal, J. F. (1984) Random digit dialing selecting a population-based control group. *Am. J. Epidemiol.* 120: 825–833.
- Kristal, A. R., Feng, Z., Coates, R. J. & George, V. (1997) Associations of race/ethnicity, education, and dietary intervention with the validity and reliability of a food frequency questionnaire: The Women's Health Trial Feasibility Study in minority populations. *Am. J. Epidemiol.* 146: 856–869.
- Willett, W. C. (1998) *Nutritional Epidemiology*, 2nd ed. Oxford University Press, New York, NY.
- Franceschi, S., Favero, A., Decarli, A., Negri, E., La Vecchia, C., Ferraroni, M., Russo, A., Salvini, S., Amadori, D., Conti, E., Montella, M. & Giacosa, A. (1996) Intake of macronutrients and risk of breast cancer. *Lancet* 347: 1351–1356.
- DeStefani, E., Deneo-Pelligrini, Mendilaharsu, M. & Ronco, A. (1998) Essential fatty acids and breast cancer: a case-control study in Uruguay. *Int. J. Cancer* 76: 491–494.
- Grammatikos, S. I., Subbiah, P. V., Victor, T. A. & Miller, W. M. (1994) n-3 and n-6 Fatty acid processing and growth effects in neoplastic and non-cancerous human mammary epithelial cell lines. *Br. J. Cancer* 70:219–227.
- Greenwald, P. (1999) Role of dietary fat in the causation of breast cancer: point. *Cancer Epidemiol. Biomark. Prev.* 8: 3–7.
- Bartram, H. P., Gostner, A., Reddy, B. S., Rao, C. V., Scheppach, W., Dusel, G., Richter, A., Richter, F. & Kasper, H. (1995) Missing anti-proliferative effect of fish oil on rectal epithelium in healthy volunteers consuming a high-fat diet: potential role of the n-3:n-6 fatty acid ratio. *Eur. J. Cancer Prev.* 4: 231–237.
- Bagga, D., Capone, S., Wang, H., Heber, D., Lill, M., Chap, L. & Glaspy, J. A. (1997) Dietary modulation of omega-3/omega-6 polyunsaturated fatty acid ratios in patients with breast cancer. *J. Natl. Cancer Inst.* 89: 1123–1131.
- Burns, C. P., Haugstad, B. N., Mossman, C. J., North, J. A. & Ingraham, L. M. (1988) Membrane lipid alteration: effect on cellular uptake of mitoxanthrone. *Lipids* 23: 393–397.
- Tsai, W. S., Nagawa, H. & Muto, T. (1997) Differential effects of polyunsaturated fatty acids on chemosensitivity of NIH 3T3 cells and its trans-formants. *Int. J. Cancer* 70: 357–361.
- Stoll, B. A. (2002) n-3 Fatty acids and lipid peroxidation in breast cancer inhibition. *Br. J. Nutr.* 87: 193–198.